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THE USE OF SOLUBLE ALGINATE FILTERS

FOR THE ISOLATION OF VIRUSES (BACTERIOPHAGE)

FROM WATER

THE ONTARIO WATER RESOURCES COMMISSION

THE USE OF SOLUBLE ALGINATE FILTERS FOR THE ISOLATION OF VIRUSES (BACTERIOPHAGE) FROM WATER

A Preliminary Report

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INTRODUCTION

One of the most promising methods available for the isolation of viruses from water appears to be that employing membrane filters. The method allows the examination of relatively large volumes of water, requires no complex equipment, and uses small numbers of tissue cultures; it is thus fairly inexpensive. However, the limiting step would appear to be the elution of trapped virus from the filter, subsequent to filtration of the sample. This varies from 20 - 100%, with any given solution, for unknown reasons (Cliver, D.O., personal communication).

In the case of aluminum alginate filters, which have been successfully used in the isolation of polioviruses from swimming pool waters (1), the filter in which the virus is held may be dissolved in a very small amount of solvent. The resultant solution is harmless, both to tissue cultures and baby mice, and does not inactivate the virus itself. Concentrations of ten thousand to one are possible.

In this report, a modified method of preparation is described. The test organism used for the experiments was a bacteriophage ('phage), since, although they approximate to the size and properties of animal viruses, their detection and propagation is considerably easier.

MATERIALS AND METHODS

Filters

The preparation of the filters differs slightly from that described by Gärtner (2). A 1% (w/v) sodium alginate solution was prepared in distilled water, shaken, and allowed to stand overnight at +4°C; the viscous solution was poured into the coating applicator of a Desaga apparatus*, the clearance of which was adjusted to 0.7 mm above the glass plate. A filter paper disc (S & S 595, 11 cm diameter) soaked in electrolyte solution was placed carefully on the glass plate, avoiding air bubbles and creases. The electrolyte solution was formed by mixing 2 parts of a 0.5 N solution of lanthanum nitrate ($La(NO_3)_3 6H_2O$) in distilled water, with 1 part of 0.5 N aluminum chloride ($Al\ Cl_3 6H_2O$) in distilled water. The spreader was then drawn across the filter to deposit a thin film of sodium alginate solution. After one minute, the formation of the gel on the filter paper was complete, and the filter paper with the adherent membrane was transferred to a dish of distilled water to wash off excess salts. The membrane and support were then stored in Petri dishes lined with wet filter paper, to prevent drying and shrinkage. These 11 cm diameter membranes were subdivided to give four filters of approximately 47 mm diameter, which could be used

* Desaga apparatus - coating applicator (adjustable) and coating template tray. Canlab - 80 Jutland Rd., Toronto 18.

in the standard Millipore filter (MF) apparatus.

Cultures

The host culture of E coli B was kindly supplied by the Department of Microbiology, School of Hygiene, University of Toronto, and was maintained on nutrient agar slants, incubated at room temperature, transferred monthly. For use in the experiments, cultures were grown in nutrient broth or nutrient agar overnight at 35°C.

The 'phage used, having E coli B as its host culture, was not identified as to type, but had been isolated from raw sewage and prepared in pure stock culture, according to the method of Adams (3). The stock 'phage preparation was stored at -20°C in small amounts in vials; the titre, determined by the agar layer method (3), was 5×10^9 plaque-forming unit per ml (pfu/ml). For use, the 'phage was diluted to the required level with dilution water.

Assay

Since the agar layer method is only capable of detecting 'phage at 1 pfu/ml or greater, the low levels of 'phage used in these experiments were assayed by the most probable number method of Kott (4), with the following modification. The assay medium was lauryl tryptose broth (Difco), containing 0.15 gm/litre $CaCl_2$, added to the tubes

immediately before use; preliminary experiments had shown that this medium yielded results superior to those obtained in Kott's original medium. The presence of 'phage was detected on nutrient agar plates (nutrient broth 8.0 gm, NaCl 5.0 gm, agar 15 gm per litre).

The titre of 'phage in a sample is determined by inoculating 5 tubes containing 10 ml double strength medium with 10 ml aliquots, 5 tubes containing 10 ml single strength medium with 1 ml aliquots and 5 tubes containing 10 ml single strength medium with 0.1 ml aliquots of the sample; each tube is then inoculated with an equal amount of young (6 hour) host culture. After incubating at 35°C overnight, the presence or absence of 'phage in each tube is determined by transfer of a loopful of culture to a nutrient agar plate freshly seeded with E coli B. The number of 'phage particles in 100 ml of the original sample is then determined by applying the combination of positive tubes obtained, to the probability tables set up for the most probable number of coliforms (5) in a 100 ml water sample.

Experimental

The 'phage was diluted to give 100 - 200 particles in 200 ml tap water; a 100 ml aliquot was filtered through an alginate membrane at 600 - 650 mm Hg, while the other

served as control and was assayed for 'phage content. The membrane and its filter paper support were removed from the filter apparatus and placed in a dish of distilled water. The alginate membrane was allowed to float off the support and was removed to a plastic (47 mm) Petri dish, where 2 ml of 3.8% sodium citrate in distilled water were added. After dissolution of the filter, 1 ml of the citrate solution was added to 100 ml tap water, which was assayed for 'phage.

The recoveries of 'phage are shown in Table I. When the 'phage was diluted to a level of less than 100 particles per litre, it could consistently be detected upon filtering a 100 ml aliquot.

CONCLUSION

A modified method of preparation of soluble aluminum alginate filters is described. These filters readily enabled the recovery of 'phage from artificially contaminated tap water, at least as low as 0.1 particle/ml. At least 70% recovery was possible when 'phage was present in higher concentrations.

The size of filter used here employs the standard MF apparatus, and filtration of 100 ml samples could be achieved in about 15 minutes at 600 - 650 mm Hg negative pressure. For larger water samples, a filter of greater diameter would probably be required to shorten the filtration time.

Similar recoveries would be expected for low numbers of animal viruses in water, and it is hoped to extend these studies to the examination of water and waste water samples for the presence of these agents. In this case, the sodium citrate filter solution could be directly titrated on tissue cultures.

Table I

The Recovery of E coli B 'phage from 100 ml Samples of Artificially Contaminated Tap Water, using Filtration through Soluble Alginate Filters.

'Phage added/100 ml	'Phage Recovered/100 ml
49*	34*
79	66
130	98
33	34
79	98

* assayed by the most probable number method.

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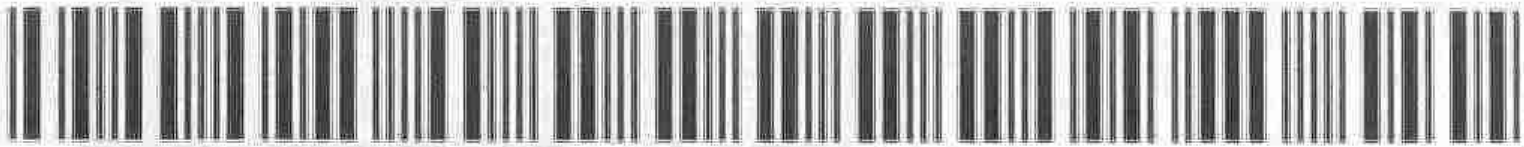
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